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Green synthesis of NiO nano-particles, phytochemical screening and antibacterial activity of aqueous leaf extract of *Jatropha gossypiifolia* (L.)

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Abstract
The importance of plant is well known. Life and its growth cannot be imagined without plants. They not only produce food for survival but also create healthy environment and eco-friendly atmosphere to live. *Jatropha gossypiifolia* (L.). Several species *J. gossypifolia* leaves with anticancer effects, anti-inflammatory and antimicrobial activity has been found to be chemo preventive. The leaf decoction of this plant is used for bathing wounds, sores, sprains, rash and bewitchment. In the recent research work, the present investigation the leaves of *J. gossypiifolia* (L.) were chemically screened for antibacterial activity by extracting them successively in various solvents such as Petroleum ether (PE). Crude extract of leaves was screened for the presence of chemically active compounds by standard methods and for antibacterial activity by zone of inhibition (mm). In my present field, a new innovative idea is discovered by promoting the synthesis of nickel particle and by phytochemical screening from the aqueous leaf extract of this plant species. The results revealed the presence of Alkaloids (AL), Saponin (SA), Tannin (TA), Flavonoid (FL), Terpenoids (TT). A new drug is to be identified and made by this leaf extract for curing diseases by carrying out the experimental analysis from the field of Nano Science and Technology. Additionally, it can be identify trace elements in the materials by XRD (X-Ray Diffraction), SEM (Scanning electron microscope), FTIR (Fourier-transform infrared) spectroscopy and UV-Visible (Ultra violet-Visible) spectroscopy and EDAX (Energy Dispersive X-Ray Analysis).

Keywords: Antioxidant nutrients, blood purifier, *J gossypiifolia*, cardio vascular disease, diabetes, cancer, sprains, FTIR, UV-Visible, XRD

Introduction
India possesses a variety of medicinal plants and it is one of the richest countries in the world in regard to genetic resources of medicinal plants. India exhibits a wide range in topography and climate, which bears varietal emporium of vegetation and floristic composition (Ravi *et al*., 2004) [1]. Moreover, the agro-climatic conditions are favorable for introduction and domestication of new exotic plant varieties. Since time immemorial, human beings have depended on nature for their simple requirements as being the sources for medicines, shelters, food, stuff, fragrances, clothing, flavors, fertilizers and mean of transportation throughout the ages (Morton, 1987; Reynertson *et al*., 2005) [2, 3]. The fundamental of typical traditional system of medicine used for thousands of years that have been in existence have formed from plants. The plants are remaining to offer mankind with new medicine. Some of the beneficial properties ascribed to plants have recognized to be flawed and medicinal plant treatment is based on the experimental findings of hundreds to thousands of years. The earliest reports of carved on the clay tablets in cuneiform date from about 2600 BC are from Mesopotamia; among the materials that were oils 9 Ravi *et al*., 2004 a) [4]. It is still used today for the cure of diseases extending from colds and coughs to inflammation and parasitic infections. Man has used various parts of plants in the treatment and prevention of various ailments. In recent years, secondary plant metabolites (phytochemicals), previously with unknown pharmacological activities, have been extensively investigated as a source of medicinal agents (Mahmoud *et al*., 2001; [5, 6]. Thus, it is anticipated that phytochemicals with adequate antibacterial efficacy will be used for the treatment of bacterial infections in near future.
The foundations of modern pharmaceutical industry were laid when techniques were developed to produce synthetic replacements for many of the medicines that had been derived from the forests (Hartnoll, *et al*., 1993) [7]. The genus *Jatropha* belongs to tribe *Joanneasiae* of *Crotonoideae* in the *Euphorbiaceae* family and contains approximately 175 species, cultivated

throughout the tropical to temperate regions of the world, have been used in different ailments like bleeding, cancer, diarrhea, fever, pain and infection, jaundice, useful in chronic dysentery, thirst, tridosha, urinary discharges, abdominal complaints, biliousness, anemia, fistula, and diseases of the heart (Li-Ming Bao *et al*., 2012; [8, 9]. Homeopathically it is used for cold sweats, colic, collapse cramps, cyanosis, diarrhea, and leg cramps. The root, stem, leaves, fruit, seed, bark and latex of the plant are largely used for the treatment of many diseases in different parts of the world. In the present study [10], we discussed the medicinal uses and chemical constituents of some of the species of *Jatropha*. "The young leaves may be safely eaten, steamed or stewed". They are favored for cooking with goat meat, said to counteract the peculiar smell. Though purgative, the nuts are sometimes roasted and dangerously eaten (Niemann *et al*., 2005; Gosemann *et al*., 2005) [11, 12]. In India, pounded leaves are applied near horses "eyes to repel flies". The oil has been in use for illumination, soap, candles, adulteration of olive oil, and making Turkey red oil. It is reported to be abortifacient, anodyne, antiseptic, cicatrizant, depurative, diuretic, emetic, hemostat, lactogogue, narcotic, purgative, rubefacient, styptic, vermifuge, and vulnerary (Jie Chen and Lyn Craven) [13].

Materials and Methods

Plant material

Jatropha gossypiifolia (L.) leaf was collected from Bavani River during the month of December 2019 (Figure one).

Fig 1: A-G: *Jatropha gossypiifolia* (L.) habitat Fig A and B: Habit; Fig C: Leaf; Fig D: Flower; Fig E: Seed; Fig F: Stem; Fig G: Root system

Preparation of the extract

Fresh leaves of *J. gossypiifolia* were harvested and washed with distilled water so as to remove dust and other foreign particles. The leaves were then left on a clean surface to dry well. The leaves were air-dried under shade for 6 - 8 days. Then the dried material was grinded to fine powder using an electric grinder and stored in air tight bottles. The powdered material was used further for phytochemical screening and preparation of extracts. The voucher specimen of this plant sample related information is preserved for future reference in the Department of Chemistry, Vivekanandha College of Arts and Sciences for Women (Autonomous), Elayampalayam, Tiruchengode, Namakkal District, Tamil Nadu, India. The Nickel oxide composite was prepared by direct chemical precipitation of nickel nitrate on active carbon from 3g $Ni(No_3)_2$ and it is dissolve in the 50ml of water in the 400ml beaker.

Maceration process

Maceration process was conducted with the pellet by magnetic stirrer (a). 9g of KOH pellets was dissolve in the 50ml of water and it is slowly dropped into the above mixed solution, and precipitation is form slowly; the molar ratio of $Ni(No_3)_2$/KOH was 3: 9. The temperature of the precipitation process was about 350 °C. The mixed solution was stirred for 4 hours. And above content was filtered with the No.1 Whatman filter paper by washing with acetone and dried it for 60-80 °C in the hot air oven (b) for 4 hours. Then precipitated material was transfer into the silica crucible and heated at 400 °C in the muffle furnace (c) for 4 hours. And the silica crucible was kept in the desiccators for cooling. The composite were well grind in fine powder particles with the help of mortar and pestle (d). The fine powder particles is washed with the ethanol, after the evaporation of solvent ethanol, the phase composition of the products were Characterized by an XRD (X - Ray Diffraction); SEM (Scanning Electron Microscope); FT - IR (Fourier Transform Infrared Spectroscopy): UV - Visible (Ultra Violet -Visible Spectroscopy).

Identification of certain biologically active compounds

Fresh leaves of *J. gossypiifolia* were washed and chemically screened to find out the presence of Alkaloids (AL),

Flavonoids (FL), Terpenoids (TR), Glycosides (GL), Tannins (TA), Saponins (SA) and Phenolic (PH) compounds.

Extraction

The dried plant material was pulverized into fine powder using a grinder (mixer). 6g crude powder of *J. gossypiifolia* leaves part was dissolve in 60 - 80ml of water in the 400ml beaker. The beaker was heated for 20 - 25mins in the Bunsen burner. Take 14.5g Nickel nitrate and it is dissolve in 100ml of water. Maceration process was conducted in magnetic stirrer (a). From the hot extract of crude powder, take 10ml and slowly dropped into the above mixed solution. Then the temperature of precipitation process was about 350 °C. The mixed content was stirred for 4 hours. And the content was filtered with the No.1 whatman filter paper and the extract in the watch glass; the sticky greenish-brown substances were obtained and stored in desiccators for further use. Then it dried for 1 hour in room temperature. The dried substances were washed thoroughly with ethanol and grind well with the help of mortar and pestle (d). Some of the extracts of each solvent were used for the qualitative phytochemical screening, then identification of the various classes of phase composition of the products were characterized.

Preliminary phytochemical screening

Qualitative phytochemical analysis of the crude powder of the leaves collected was determined according to the standard procedures to identify the constituents as described by. Foam test for saponins, Salkowski and Liebermann-Burchard test for terpenoids and triterpenoids, Ferric chloride test ($FeCl_3$) test for tannins, Keller-Killiani test for cardiac glycosides, Fehling's test for reducing sugars, xanthoproteic test for proteins, iodine test for starch and ammonia test for detection of flavonoids were performed to identify the constituents present in the extracts of the leaves of the plant.

Results and Discussion

Phytochemical screening test of *Jatropha gossypiifolia* leaves extract

Plants are the great importance to the health of individuals and communities from time immemorial. Plant kingdom provides a tremendous reservoir of various phytochemicals with potential therapeutic properties. The major phytochemicals of interest are alkaloids, tannins, flavonoids, phenolic compounds, steroidal sapogenins (saponins); however, other diverse groups of naturally occurring phytochemicals such as unsaturated sterols, triterpenois, and essential oils are also present. These phytochemicals play important role in herbivore deterrence due to astringency or they may act as phytoalexins, killing bacteria that the plant recognizes as a threat. Plant extracts are used to treat numerous human diseases and have prominent effect on the animal system, important therapeutic properties and antimicrobial activities against various pathogens. Plant can function as sources of anti-cancer agents. Percent extractive value of various fractions of partially purified hot extracts is depicted. Maximum percent extractive value was observed for aqueous fraction (Table 1).

Table 1: Extractive of different partially purified fractions of *J. gossypiifolia* leaves extract

S. No.	Solvent	Percent extractive value
1.	Petroleum ether Fraction (PEF)	4.9
2.	Benzene Fraction (BEF)	1.72
3.	Chloroform fraction (CHF)	2.37
4.	Acetone fraction (ACF)	1.4
5.	Ethanol fraction (ETF)	4.5
6.	Methanol fraction (MEF)	3.37
7.	Aqueous fraction (AQF)	6.65

All the tested phytochemicals were found to be present in dried plant material (leaves) as shown in (Table 2). The presence of flavonoids and tannins in the leaves is likely to be responsible for the free radical scavenging activity. Flavonoids and tannins are phenolic compounds and plant phenolics are a major group of compounds that act as primary antioxidants or free radical scavengers. These findings give credence to the traditional medicinal application of the leaves as remedies for sores, rash and bewitchment, internal and external wounds and infections. Flavonoids have been referred to as nature's biological response modifiers because of strong experimental evidence of their ability to modify the body's reaction to allergies, virus and carcinogens. They show anti-allergic, anti-inflammatory, antimicrobial and anti-cancer activity. Cardiac are used in the treatment of congestive heart failure and cardiac arrhythmia. They are also, used to strengthen a weakened heart and allow it to function more efficiently. Steroids anti-inflammatory effects. Glycosides, flavonoids, tannins have hypoglycemic activities. Saponins possess hypocholesterolemic and antidiabetic properties. The terpenoids have also been shown to decrease blood sugar level in animal studies. Steroids and triterpenoids showed the analgesic properties. The steroids and saponins are responsible for central nervous system activities.

Table 2: Qualitative phytochemical screening of *J. gossypiifolia* leaves

JTS	Test	Presence/Absence
1.	Alkaloids (Drangendroff's Test)	+
2.	Phenolic compounds ($PbCH_3COO$ Test)	+
3.	Flavonoids (Shinoda's Test)	+
4.	Glycosides (Killer Kiliyani's Test)	+
5.	Terpenoids (Salkowski Test)	+
6.	Tannins ($FeCl_3$ Test)	+
7.	Saponins (Frothing Test)	+

X-ray diffraction (XRD): X-Ray Diffraction is a powerful, non-destructive technique for characterizing crystalline materials. It provides information on structures, phases, preferred crystal orientation and other structural parameters such as average gain size, crystallinity and crystal defects. The characteristic x-ray diffraction pattern generated in a typical XRD analysis provides a unique "fingerprint" of the crystals present in the sample. When properly interpreted, by comparison with standard reference patterns, this fingerprint allows identification of the crystalline form. The particle size of the prepared samples were determined by using Scherrer's equation as follows $D \approx 0.9\lambda\beta\cos\theta$ where D is the crystal size, λ is the wavelength of X-ray, θ is the Braggs angle in radians and B is the full width at half maximum of the peak in radians.

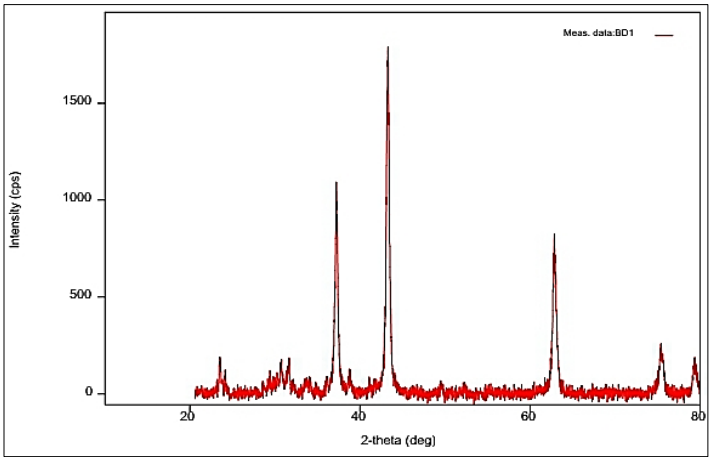

Fig 2: XRD spectrum of NiO nanoparticles

Calculate to find the nano particle size by using Scherrer's equation

$$\frac{42.39 + 106.10 + 14.11 + 61.63 + 17.28 + 12.05 + 48.51 + 24.33 + 31.43 + 24.20 + 70.33 + 21.86 + 20.99 + 22.01}{14}$$

$$= \frac{517.22}{14}$$

$$= 36.9443 \text{ nm}$$

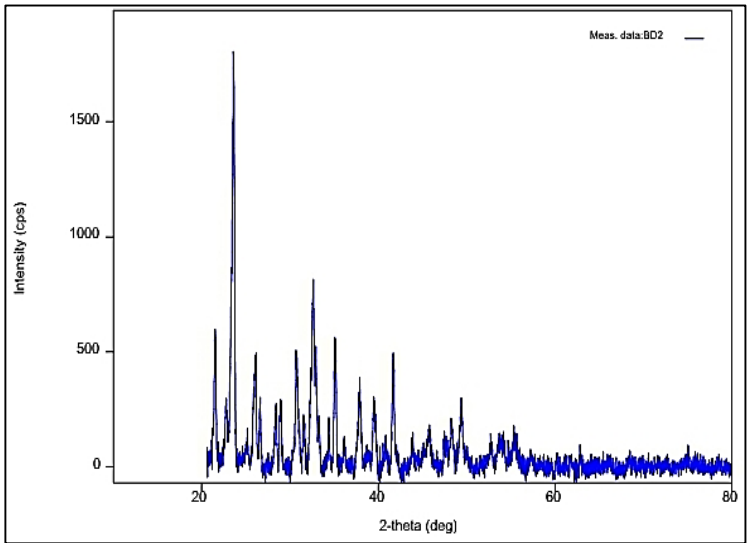

Fig 3: XRD spectrum of NiO nanoparticles using *Jatropha gossypiifolia* leaf extract

Calculate to find the phytoparticles size by using Scherrer's equation

$$26.93 + 26.04 + 33.30 + 36.51 + 17.56 + 36.11 + 36.82 + 24.16 + 16.35 + 22.90 + 38.77 + 33.06 + 34.99 + 21.79 + 37.50 + 13.81 + 66.64 + 144.82 + 34.01 + 43.65 + 22.50 + 28.39 + 25.20 + 28.39 + 25.20 + 30.55 + 37.96 + 47.09 + 18.77 + 24.53 +$$

$$= \frac{23.33 + 21.25 + 32.71 + 34.32 + 11.80 + 16.46 + 47.31}{35}$$

$$= \frac{1167.89}{35}$$

$$= 33.3682857 \text{ nm}$$

Fourier transform infrared spectroscopy (FT-IR)

The interaction of surface biological group with the metal can be studied by the valuable analytical tool FT-IR. The sample is characterized by FT-IR within the wavelength range of 800-20000 cm^{-1}. Infrared spectroscopy is an important technique inorganic chemistry. It is an easy way to identify the presence of certain functional group in a molecule. This technique was applied to determine the group that was present in the surface of nanoparticles. The FT-IR spectrum of NiO nanoparticles exhibit peaks at 1570 cm^{-1}, 1370 cm^{-1}, 1100 cm^{-1}, 942 cm^{-1}, 884 cm^{-1}, 844 cm^{-1} and 803 cm^{-1}. A peak at 1570 cm-1 corresponds to N-O stretching (Ashokkumar and Ramaswamy, 2014) [14].

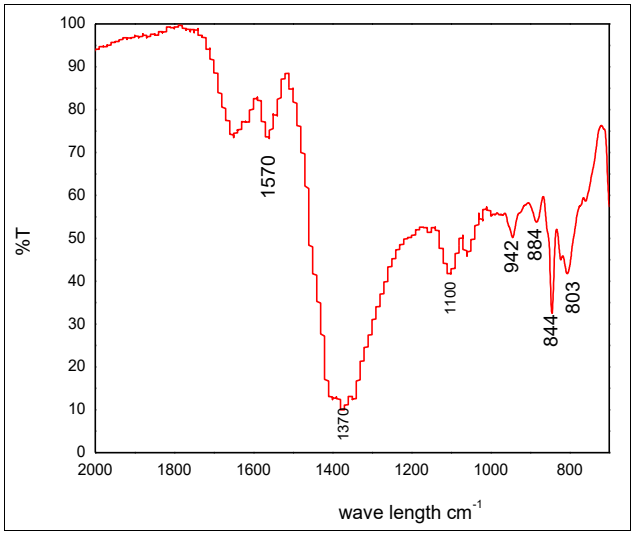

Fig 4: FT-IR spectrum of NiO nanoparticles using *Jatropha gossypiifolia* leaf extract

A vibration at 1370 cm-1 corresponds to C-H bending methyl. A peak at 1570 cm-1 corresponds N-O stretching. A peak at 1100 cm- 1 corresponds to C-O stretching of Carbonyl. The bond appeared at 803cm-1 could be assigned for the C-H bending vibration.

Phytochemical test

Phytochemical analysis was done by referring standard protocol 6,12,3. The major phytochemicals of interest are alkaloids, tannins, flavonoids, phenolic compounds, steroidal sapogenins (saponins); however, other diverse groups of naturally occurring phytochemicals such as unsaturated sterols, triterpenois, and essential oils are also present.

Preliminary phytochemical screening

The condensed extracts of different solvent used for preliminary phytochemical screening were carried out using standard procedures to test the presence of bioactive compounds (Amarasingham *et al*., 1964) [15], (Chabra *et al*., 1984) [16], Harborne (1984) [17].

Fig 5: Phytochemical screening test

Qualitative and quantative analysis

Various chemical constituents have been detected in extracts from different parts of *J. gossypiifolia*, the literature having reported, in general, the presence of fatty acids, sugars, alkaloids, amino acids, coumarins, steroids, flavonoids, lignans, proteins, saponins, tannins, and terpenoids. Accordingly reviewed by Zhang *et al*. the main compounds isolated from *Jatropha* genus are the terpenoids. In fact, many of them were isolated from different parts of *J. gossypiifolia*. Another very important class from *J. gossypiifolia*is the lignoids, since a good number of them was already isolated and identified. However, it is important to note that most of the phytochemical studies found in literature are not about isolation of compounds, but only about the phytochemical screening of the major classes through chemical qualitative reactions or more sensitive and specific methods such as thin

layer chromatography (TLC). Relative to other *Jatropha* species, few studies have isolated chemical compounds from *J. gossypiifolia*. In addition, up till now it is not clear which are the major bioactive compounds in the plant, since only a few studies were conducted by bioassay-guided isolation. Additionally, to the best of our knowledge, there are no phytochemical studies regarding the use of water as solvent for the extraction of *J. gossypiifolia* constituents. This is important to note since popular use occurs more frequently with infusions or decoctions, and little is known about the constitution of this type of extract. In this context, it is important to conduct studies to evaluate the phytochemical constitution of these extracts. More commonly, the studies use solvents or mixtures of solvents with nonpolar characteristics, which could contribute to further characterization of nonpolar compounds, such as terpenoids and lignoids. Polar compounds such as flavonoids, tannins, and sugars are poorly described in the species so far, probably due to this fact.

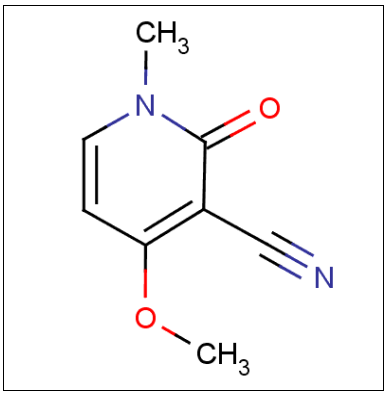

Fig 6: Structure of ricinine

Isolated *ricinine* from the ethyl acetate extract from senescent leaves, the main compound responsible for the toxicity of the crude extract in Spodopteraexigua larvae, thus demonstrating that it could be an alternative choice to chemical insecticides (Vasakorn Bullangpoti *et al.*, 2011) [18]. In another study, (Vasakorn Bullangpoti *et al.*, 2011) [18] showed that the ethanol extract of *J. gossypiifolia*in association with the ethanol extract of *Melia azedarach* was toxic and inhibited some enzymes from Spodoptera frugiperda larvae, demonstrating once more the potentiality of the species as insecticide agent. (Calatayud *et al*. 2011) [20] showed the presence of proteins of about 100 kDa with toxic activity upon Phenacoccusherreni, another type of insect. In this work, the authors performed a strategy of extraction that eliminated non-protein compounds, being able to demonstrate the potential of the species to obtain insecticidal proteins. Leaf extract of *J. gossypiifolia* reduced the fecundity and egg viability against stored product insect pests Triboliumcastaneum. The potential molluscicidal activity of *J. gossypiifolia* has also been evaluated as an alternative mode of prevention of schistosomiasis. Sukumarn *et al*. showed that the methanol and n-butanol extracts from unripened seeds of *J. gossypiifolia*was toxic against eggs and adults of two species of freshwater snails, *Lymnae aluteola* and Indoplanorbisexustus (Vasakorn Bullangpoti *et al.*, 2011) [18]. The results indicated that n-butanol extract was the most effective and that the eggs were more susceptible than adults.

Assays for antibacterial activity

Bacterial strains of *Staphylococcus aureus, Bacillus subtilis, Bacillus cereus* and *Klebisella* were rocured from Department of Microbiology, Vivekanandha College of Arts and Sciences for Women (Autonomous), Tiruchengode. Disc diffusion method (Bauer *et al*., 1996) [19] was used to assess the effect of plant extracts on bacteria. The plant extract were added at concentration of 2.5, 5, and 10% (dissolved in respective solvents) to the aseptic medium and agar plates were prepared. All the tested microorganisms were grown previously in the same agar plates individually. Disc without plant used as control. The plates were left for aerobic incubation at 30ºC for 16 hrs. and growth of the organisms was observed in treatments. Presence (+) or absence (-) of antibacterial activity and the size (diameter) of the inhibition zones was also need in respective of solvent.

Antibacterial activity of NiO Nanoparticles prepared by using *Jatropha Gossypiifolia* leaves extract. Antibacterial activity of NiO nanoparticles were carried out to test the bacterial efficiency of the nanoparticles. It has demonstrated that highly reactive metal oxide nanoparticles exhibit excellent biocidal action against gram positive and gram negative bacteria. The toxicity is depends on both the exposure and size of metal nanoparticle. The test was carried out using different concentrations of the nanoparticles by disc diffusion method. NiO nanoparticles exhibit higher zone of inhibition against the bacterial pathogens such as *Staphylococcus aureus, Bacillus subtilis, Bacillus cereus* and *Klebisella*.

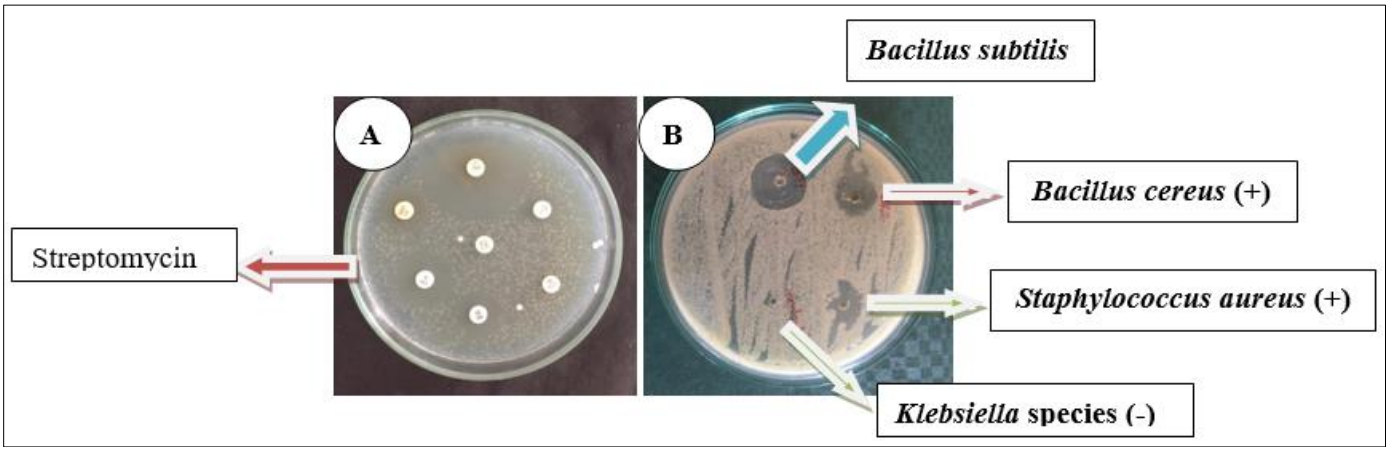

Fig 7: A-D: Antibacterial activity of NiO nanoparticles

Table 3: Antibacterial activity of chemical name using *streptomycin* as a standard drug - zone of inhibition

Compound	Name of microorganisms employed			
	Gram positive bacteria (+) (mm) (Zone of inhibition (mm)			Gram negative bacteria (-) (Zone of inhibition (mm)
	Bacillus subtilis	***Bacillus cereus***	***Staphylococcus aureus***	***Klebsiella* species**
Nanoparticles	4.1	3.6	6.2	3.5
Streptomycin (Antibiotics)	5.9	4.9	5.2	5.3

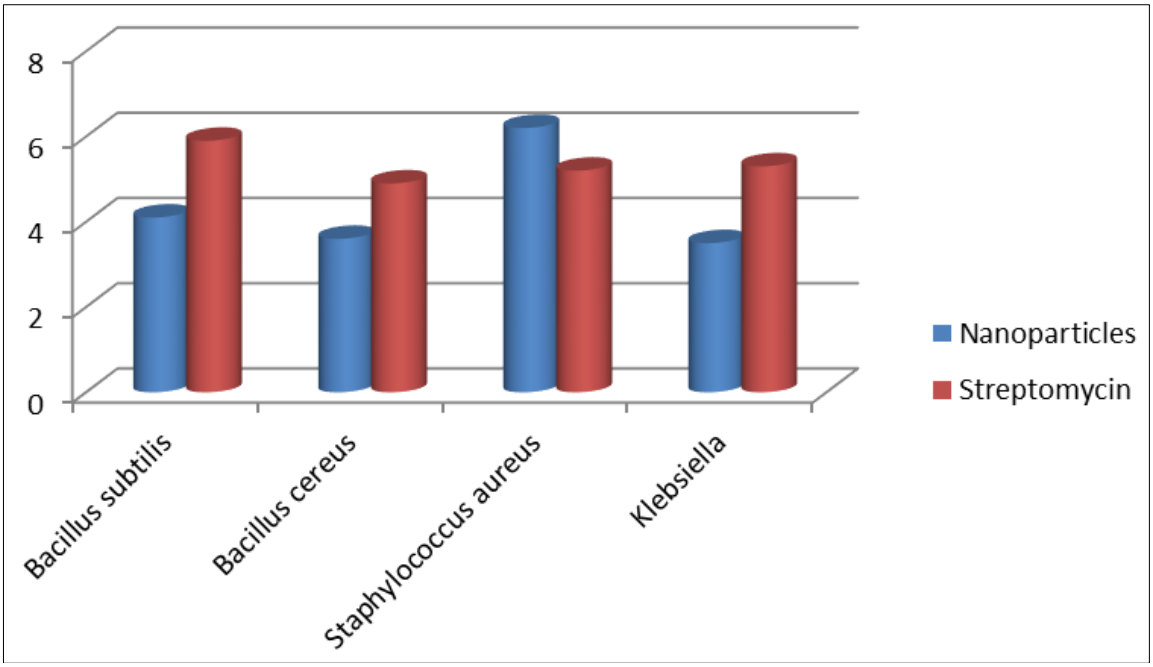

Fig 8: Antibacterial activity of chemical name using ciprofloxacin as a standard drug - zone of inhibition (mm)

Table 4: Antibacterial activity of chemical name using ciprofloxacin as a standard drug – zone of inhibition

Compound name	Name of microorganisms employed			
	Gram positive bacteria (+) (Zone of inhibition (mm))			Gram negative bacteria (-) (Zone of inhibition (mm))
	Bacillus subtilis	***Bacillus cereus***	***Staphylococcus aureus***	***Klebsiella* species**
Phytoparticles	4.3	4.6	5.2	4.5
Ciprofloxacin	5.9	3.9	4.2	4.3

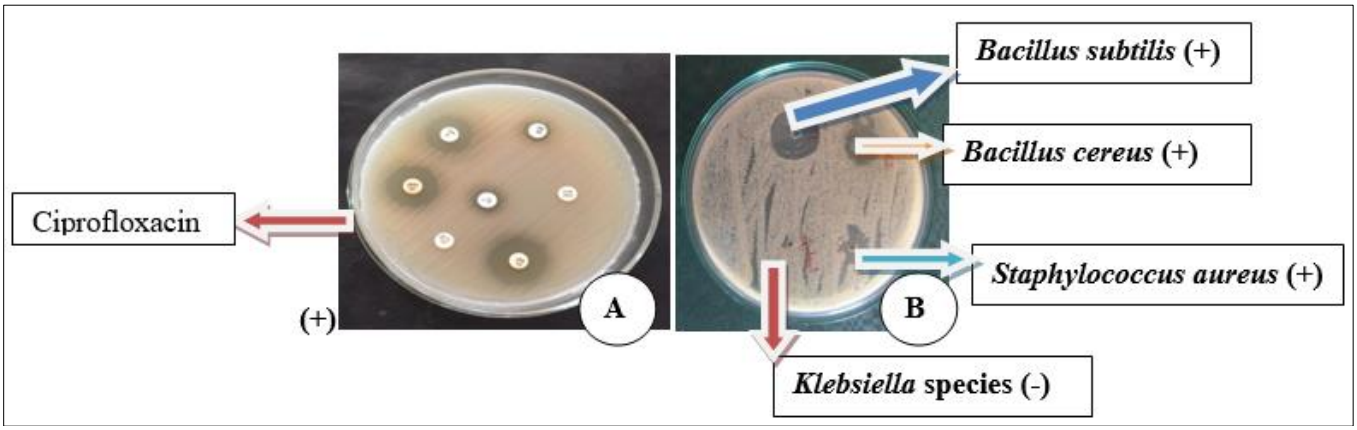

Fig 9: Show the A and b Antibacterial activity

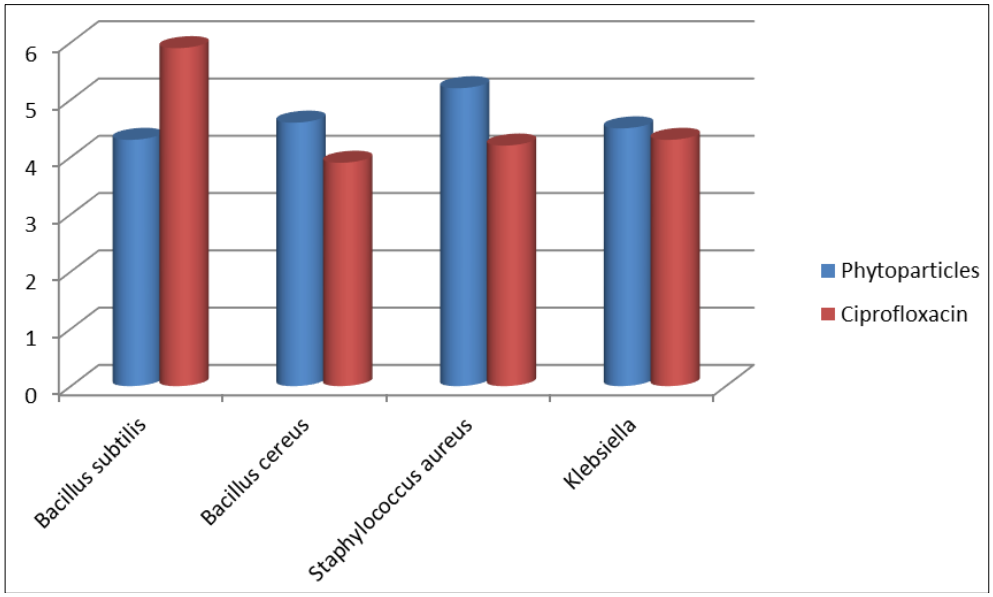

Fig 10: Antibacterial activity of chemical name using ciprofloxacin as a standard drug zone of inhibition in mm

Conclusion

The development of novel compounds with phytochemical activity is an urgent need. In this work, we used easily available, inexpensive starting materials and procedure is easy to carry out in any laboratory. Use of toxic reagents is avoided. Here we have reported eco-friendly synthesis of NiO nanoparticles using *Jatropha gossypiifolia* leaf extract. It has been used as reducing agent and also a capping agent in the NiO nanoparticle synthesis. This process was completely undertaken through green synthesis route. The freshly prepared leaf extract was added to 14.5g Nickel nitrate and the reaction takes place at room temperature. The synthesized nanoparticles were of rod shaped and the particles sizes were 30-40nm. In this study, green synthesis methods were pollutant free and eco-friendly. The traditional medicine of *Jatropha gossypiifolia* extract with Nickel nitrate results in the formation of Nickel oxide nanoparticles. Nickel oxide nanoparticles characterized by UV-VIS, FT-IR, XRD, SEM, and EDAX. Further, these synthesized NiO nanoparticles from *Jatropha gossypiifolia* shows antibacterial activity on both gram positive and gram negative bacteria which could be excellent antibacterial agent to treat diseases caused by bacteria.

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